STUDY OF POSSIBLE MECHANISM UNDERLYING IN NEUROPROTECTIVE POTENTIAL OF URSOLIC ACID AND MAGNOLOL IN 3-NITROPROPRIONIC ACID INDUCED HUNTINGTON DISEASE IN ANIMAL MODEL

SYNOPSIS

Submitted in partial fulfillment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY
in
Pharmacy

Submitted by

Ravinder Khatri
Enrollment No.: H220261

Under the supervision of

Dr. Kamal Jeet
Assistant Professor

DEPARTMENT OF PHARMACY
SCHOOL OF PHARMACEUTICAL AND HEALTH SCIENCES
CAREER POINT UNIVERSITY HAMIRPUR
TIKKER (KHARWARIAN), BHORANJ
HAMIRPUR (HP)-176041, INDIA

2022-23

INTRODUCTION

Over 50 million people worldwide suffer from neurodegenerative illnesses. In the domains of study and medicine, it is still a mystery, and for the most of them, there is no long-term treatment. But, there is still hope because to persistent soul searching, modern therapies and rehabilitation programmes that reduce symptoms, boost mobility, and improve the quality of life for patients and their families (Checkoway, 2011)

Types of Degenerative Nerve Diseases Include:

- Alzheimer's disease
- Amyotrophic lateral sclerosis
- Friedreich ataxia
- Huntington's disease
- Lewy body disease
- Parkinson's disease
- Spinal muscular atrophy

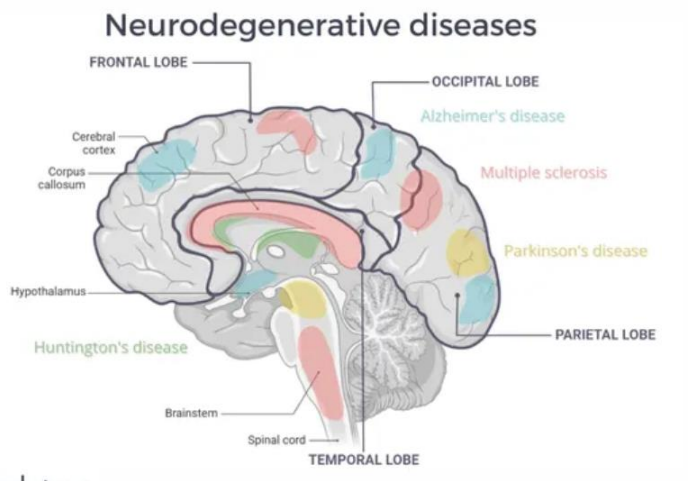

Figure 1: Longitudinal section of brain

Numerous body functions, such as self control, mobility, talking, respiration, and cardiovascular function, are affected by diseases of the degenerative nerve. When nerve cells in the brain or peripheral nervous system start to operate less effectively over time and eventually perish, this happens. Many of these diseases have inherited traits. It is occasionally possible for a disease like alcoholism, a cancerous tumour, or a stroke to be the cause of the issue. Viruses, chemicals, and toxins are among additional potential reasons. There are times when no cause is known.

The human brain has an estimated 100 billion neurons involved in learning and memory processing (Herculano-Houzel, 2009). Loss or deregulation of transmitter release, oxidative stress, and inflammation affect neuronal plasticity and escort to cognitive impairments (memory loss) and loss of body weight involving the brain regions include basal ganglia, cerebral cortex and hippocampus, and depressive symptoms noticeable in huntington and in mental deterioration and progressively worsening chorea is associated with huntington’s diseases (HD) (Khan *et al*., 2015; Wang *et al*., 2017). HD was firstly described in 1872 by the American physician George Huntington. The onset of HD is typically in middle age approximately at 40 years.

The neurodegenerative process primarily involves medium spiny striatal neurons (MSN) and to a lesser extent cortical neurons. MSNs (D_1-MSN) express D_1 dopaminergic and M_4 muscarinic receptors which project into the internal segment of globus pallidus (GPi) and substantia nigra (SN). Striatopallidal MSNs (D_2-MSN) express D_2 dopamine and adenosine A_2A receptors and project into the external segment of globus pallidus (GPe). It is well established that loss of GABAergic MSNs in striatum is responsible for hyperkinetic movements in HD (Do *et al*., 2013). Beside this many other factors, which can trigger cognitive loss are tumors of brain, stroke (Lee *et al*., 2012), actinotherapy (Kushner, 1998), intracranial damage, migraine cephalagia, heat stroke (Uzun *et al*., 2012), medications such as benzodiazepines (Hermann and Seidenberg, 2007), epilepsy (Luchsinger and Mayeux, 2004), diseases like, HIV/AIDS, Lyme or Syphilis, nutrient and vitamin deficiencies such a lack of vitamin B_{12} or thiamine, and excessive alcohol use can all affect the neurological system (Morris *et al*., 2007).

HD is caused by a mutation encoding an abnormal expansion of CAG encoded polyglutamine repeats in a protein called huntingtin (htt). The HD gene is localized on chromosome 4 more specifically, chromosome 4p16.3 and comprises 67 exons and 3144 amino acids (Collaborative Research Group, 1993). In transgenic mice expressing human huntingtin with an expanded CAG/polyglutamine, a progressive syndrome develops, which is characteristic of human HD (Mangiarini *et al*., 1996). The protein huntingtin (htt) is widely expressed within the central nervous system and in extra neural tissues. Huntingtin is expressed more intensely in neurons than in glial cells (Bradford *et al*., 2010).

Accumulation of proteolytic htt fragments and their aggregation trigger a cascade that leads to increasing neuronal dysfunction through oxidative injury and glutamate excitotoxicity (Mark *et al*., 2001; Kim *et al*., 2015), transcriptional deregulation (Basso, 2012), apoptotic signals, mitochondrial dysfunction (Johri and Beal, 2012; Costa and Scorrano, 2012; Chaturvedi and Beal, 2013) and energy depletion (Ribeiro *et at*., 2012). These changes are accompanied by neuro-chemical alterations, which involve not only GABA receptors but also other receptors, such as the glutamate, dopamine (DA) and adenosine receptors involved in motor functions (Ellerby, 2002; Chen *et al*., 2013). The age of onset and disease severity are dictated by the extent of the HD gene mutation and by the sex of the patient. However, environmental factors and genetic modifiers can modify the variability of clinical expression of Huntington's disease (Gusella and MacDonald, 2009).

Evidences suggests that glutamatergic excitotoxicity in striatum is predominantly mediated by the over activation of N-methyl-D-aspartate (NMDA) receptor particularly through NR_2B subunit (Milnerwood *et al*., 2010).

To investigate the mechanism of neurodegeneration in HD, experimental animal models of HD have been generated using various excitotoxins, neurotoxins and genetic manipulations. Injections of NMDA receptor agonists, such as quinolinic acid, into the striatum, induce HD-like pathology, with a loss of projecting MSN and sparing of cholinergic and nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase neurons (Beal and Ferrante, 2004).

Inducing Agent (3-Nitroproprionic acid)

Peripheral injections into rodents or primates of several mitochondrial toxins, including 3-nitroproprionic acid (3-NP), also reproduce the aspects of behavioral, biochemical and striatal pathological alterations found in HD (Dhir *et al*., 2008; Kumar and Kumar, 2009) 3-NP, a mycotoxin, is a suicidal irreversible inhibitor of succinate dehydrogenase (SDH), enzyme located in mitochondrial inner membrane (Biniendaa *et al*., 2010; Binawade and Jagtap 2013; Zwolak, 2014) a member of both Kreb's cycle (oxidizing succinate to fumarate) and an entry point for electrons into the respiratory chain at the level of ubiquinol (Huang, 2006). Inhibition of SDH interferes with the electron cascade and interrupts oxidative phosphorylation. This phenomenon leads to reduced ATP synthesis and responsible for the initiation of oxidative stress (Shivasharan *et al*., 2013; Hariharan *et al*., 2014). Growing body of evidences explore the involvement of impaired energy metabolism, excitotoxicity, microglial over activation and production of pro-inflammatory cytokines leading to neuronal cell death, by both necrosis and apoptosis (Kim *et al*., 2002; Huitron *et al*., 2013). These events of neurodegeneration are relevant to the striatal cell loss seen in HD and are gaining prominence for 3-NP lesions (Pandey, 2009; Swaroop *et al*., 2014). Elevation of cAMP causes both short and long-term increase in synaptic strength (Kendel, 2022) and stimulates cholinergic cells to release acetylcholine (Vogelsberg and Neff, 1997). However, the levels of cAMP are reported to be decreased in neuropathological conditions (Nagakura *et al*., 2002; Puzzo *et al*., 2005). Introduction Role of cAMP mediated CREB activation in 3-NP induced neurotoxicity in rats. Further, cAMP dependent CREB phosphorylation has too been reported to induce long term memory (LTP) (Carlezon *et al*., 2005) and inhibit apoptotic and necrotic cell

death (Zhang *et al*., 2008). CREB is a transcriptional factor responsible for synthesis of proteins which are important for the growth and development of synaptic connections and increase in synaptic strength (Benito and Barco 2010; Bitner, 2012). Thus, agents that enhance cAMP/PKA/CREB pathways have potential for the treatment of stroke and other neurological diseases (Mehan *et al*., 2010). An antioxidant molecule is crucial to the protection of cells against the development of prooxidantstate. Antioxidants may protect against reactive oxygen species (ROS) toxicity (Sen, 1995) by means of:

- Prevention of ROS formation, Interruption of ROS attack.
- Scavenging the reactive metabolites and converting them to less reactive molecules.
- Enhancing the resistance of sensitive biological targets to ROS attack.
- Facilitating the repair caused by ROS.
- Providing co-factors for the effective functioning of other antioxidants

LITERATURE REVIEW

Ursolic Acid (UA)

Solomon Habtemariam (2019) studied that the plant triterpenoid a pentacyclic molecule, 3β-hydroxy-12-urs-2-en-28-oic acid is known as ursolic acid (UA). Common herbs and medicinal plants contain UA, by reacting with free acid or triterpenoid saponins to generate an aglycone, ursolic acid is transported throughout plants.

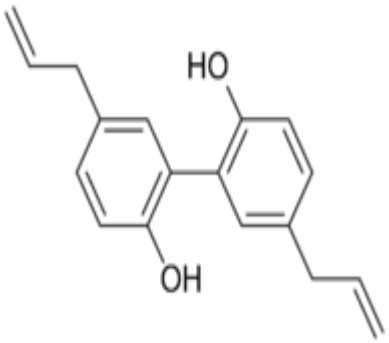

Figure 2: Ursolic Acid

Lopez-Hortas (2018) wrote that the UA triterpenoid is well documented to be available in fruits such as *Vaccinium macrocarpon* (cranberries), Vaccinium spp. (blueberries), *Ocimum basilicum* (basil), *Olea europaea* (olive), *Origanum vulgare*, *Rosmarinus officinalis*, Salvia, and Thyme (thyme), *Calluna vulgaris* (heather flower), *Pyrus pyrifolia* (pear), *Ledum groenlandicum*, Retzius (Labrador tea), *Rosmarinus officinalis* (rosemary) and *Malus pumila* (apple peels) which possess important health benefits. UA is also distributed among higher plants, such as *Mimusops caffra*, *Ilex paraguarieni*, *Glechoma hederaceaes*, *Ichnocarpus frutescens*, and *Syzygium claviflorum*. UA is still a crucial and

well-liked antioxidant agent, and numerous findings indicate that it may protect the brain from ischemic stroke.

Numerous lines of research support the pharmacological effects of UA, which include neuroprotection, anti-cancer, anti-microbe, sedation, hepatoprotection, anti-inflammation, anti-oxidation, and regulating blood glucose. These effects make UA a potentially effective preventive and therapeutic agent for many diseases, such as cancer, bacterial infection, diabetes mellitus, Alzheimer's disease, immunological disease, and others.

Q.Li *et al.*, (2015) and Meng *et al.*, (2020) studied that the ursolic acid can also stimulate muscle growth, reduce fat gain and enhance the epidermal permeability barrier recovery in the skin, therefore it has been proposed as a skin therapeutic agent and it could be introduced in sport supplements, cosmetics and health products (Safayhi & Sailer, 1997). More recently, the therapeutic effects of UA were assembled along with its mechanisms of action for the treatment and prevention of health conditions

Habtemariam, (2019) employed a subarachnoid haemorrhage brain injury model in rats to study the potential effect of UA administration (25 or 50 mg/kg) over a 48 h observation period. From the inflammatory end-point measurement that they recorded, a significant reduction of the treatment group included intercellular adhesion molecule-1 (ICAM1), toll-like receptor 4 (TLR4), nuclear factor-κB (NF-κB) P65, interleukin-1β (IL-1β), tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS), and matrix metalloproteinase- (MMP) 9. These anti-inflammatory effects coupled with a reduction in the apoptosis score suggest the neuroprotective effect of the compound.

Hong *et al.*, (2021) showed the capacity of UA to decrease the production of TNF-α in RAW 267.4 and A549 cells infected with *Mycobacterium tuberculosis* and Con A-stimulated mouse splenocytes to detect anti-inflammatory activity. UA significantly inhibited the mRNA expression levels of TNF-α, IL-1β and IL-6 in the stimulated cells. The expression levels of COX-2 and iNOS were also suppressed by UA

Huang *et al.*, (2016) reported a similar study in which in vitro inhibition of cyclooxygenase-2 activity was due to active compounds (UA, cis-hydroxycinnamoyl

ursolic acid and trans-hydroxycinnamoyl ursolic acid) UA primarily prevents hepatocellular carcinoma (HCC) from growing and migrating by impairing their glucose, ester, and genetic material metabolism. In order to restore a damaged liver, UA also has the ability to encourage the regeneration of liver cells. Furthermore, UA has anti-inflammatory, antioxidant, anti-HCV/HBV, and antidiabetic properties that can help treat HCC.

Wang *et al.*, (2016), examined the biological antibacterial effects of UA against the methicillin-resistant staphylococcus aureus (MRSA) in their research. Researchers found that UA reduced the integrity of the staphylococcal membrane without causing any hemolytic effects. UA affected the translation efficiency, chaperon subunits, ribonuclease, oxidative processes, and glycolysis of a range of proteins involved in the transformation process, as demonstrated by UA-treated protein type relative to regular MRSA cells. Ursolic acid and its derivatives were examined for their least minimal inhibitory concentration against a number of microbial pathogen strains, including Staphylococcus aureus, Bacillus cereus, Shigella flexneri, a pair of strains of Escherichia coli (E. coli), Aeromonas caveae, Pseudomonas aeruginosa, and Klebsiella pneumonia. By utilizing the microdilution technique, diagnose pneumonia and Vibrio colareae. According to numerous toxicological investigations, ursolic acid has very little negative effects on healthy cells.

Magnolol

Chen *et al.*, (2011) Magnolol (6,6',7,12-tetramethoxy-2,2'-dimethyl-1-beta-berbaman), C(18)H(18)O(2), an active polyphenolic ingredient isolated from the root and stem bark of *Magnolia officinalis*, mainly used in Chinese medicine. It has been used in traditional medicine for managing the disturbances of the nervous system. The magnolol content is in the range of 2–11% of the bark's dry weight magnolol, a lignin, has been reported to have several pharmacological activities like antiepileptic activity due to its effect on synaptic and presynaptic GABA-A receptors

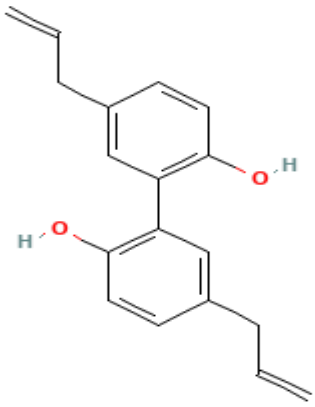

Figure 3: Magnolol

inflammatory and analgesic effect sedative-hypnotic effect. And also exhibits neuromodulatory, cognition enhancing, antimicrobial, anti-osteoporotic, anti-diabetic, anti-hyperlipidemic, anticancer, anti-inflammatory, antioxidative, neuroprotective, and anti-angiogenic activities. In a study on neuronal apoptosis in the NGF-differentiated PC_{12} cell line, honokiol and magnolol (lignans) significantly controlled Aβ induced neuronal apoptotic cell death. The neuroprotective effects are possibly mediated through a reduction in the production of Reactive Oxygen Species, decrease in intracellular calcium elevation and antagonism of caspase-3 activity.

Xian, *et al.*, (2016) Magnolol showed neuroprotective properties in cortical neuron-astrocyte cultures. (Hoi *et al*., 2010) depicted that magnolol protect against Aβ damage. While (Wang, *et al*., 1995) studied that magnolol showed anti-inflammatory activity perhaps via inhibition of cyclooxygenase and 5-lipoxygenase enzymes.

Zhu *et al.*, (2022) demonstrated the processes behind the anticonvulsant properties of magnolol (6, 6', 7, 12-tetramethoxy-2, 2'-dimethyl-1-berbaman, $C_{18}H_{18}O_2$). 30 minutes before receiving a 60 mg/kg i.p. injection of pentylenetetrazol, mice were given magnolol (20, 40, and 80 mg/kg). Magnolol's anti-seizure properties were examined utilizing behavioural seizure models, EEG, in vitro electrophysiology, and c-Fos expression in the brain and hippocampus. When compared to the vehicle-treated rats, magnolol at dosages of 40 and 80 mg/kg significantly delayed the start of myoclonic jerks and generalised clonic seizures, as well as the seizure stage and mortality. Magnolol is said to be able to pass through the blood-brain barrier (BBB) and have neuroprotective effects using a number of different pathways. Magnolol treats mental illnesses including anxiety and depression as well as physical conditions such cerebral ischemia, neuroinflammation, Alzheimer's disease, and brain tumours.

RESEARCH GAP IDENTIFICATION

Huntington's disease (HD) is a hyperkinetic movement and progressive neurodegenerative disorder that is caused by the degradation of GABA-ergic medium spiny neurons (MSNs) (Wang *et al*., 2017). An abnormal expansion of CAG in the huntingtin (Htt) gene (Iennaco *et al*., 2022). Excessive activation of NMDA receptors, increased glutamate release from cortex, and hypersensitivity of post-synaptic glutamate receptors on striatal projection neurons cause an alteration in intracellular calcium

homeostasis and mitochondrial dysfunction, which increase oxidative stress and neuroinflammation, resulting in death of striatal MSNs leading to HD (DiFiglia, 1990).

As a result of people's constant effort, aging, and oxidative stress, neurons gradually lose their structure or function, leading to neurodegenerative diseases including Huntington's and other chronic illnesses.

Ursolic acid and magnolol have been involved in a variety of physiological actions, such as antioxidants, which are effective in the prevention of numerous types of neurodegenerative disorders in which free radical processes are involved. In brief, magnolol has been found to protect the CNS from beta-amyloid toxicity, glutamate-induced excitotoxicity, nitric oxide toxicity, cyanide toxicity, and ischemia/reperfusion injury.

The effect of magnolol to neutralize the free radicals is receptor independent. Based on the investigation of the pharmacological activity of the magnolol molecule, it has been concluded that it is the reactive center for dealing with oxidants due to its highest antioxidant potential and very low activation energy barrier to free radical reactions. In recent years, many physiological properties of ursolic acid and magnolol have been described, resulting in much attention to their exploration to their fullest potential. However, the therapy of oxidative stress-related diseases has not found satisfactory application in clinical practice. This may be due to the insufficient efficacy of the drugs available and their unsuitable pharmacokinetic side effects and toxicity.

3-NP, malonate, and excitotoxins are crucial players in the progression of oxidative damage associated with HD-like lesions and are linked with mitochondrial energetic dysfunction and excitotoxicity (Tunez *et al*., 2010). Therefore, we hypothesize that targeting the oxidative stress, NMDA and glutamate receptors with their antagonists and using antioxidants to cure the HD.

The Relevance of the Proposed Study

- The MSNs of striatum contain relatively expresses high number of N-methyl-D-aspartate (NMDA) receptor and therefore are more prone to excitotoxic cell death (Milnerwood *et al*., 2010). Glutamate induced excitotoxicity is one of major

mechanism proposed for neurodegeneration in CNS disorders like HD (Mehta *et al.*, 2013).

- The existing HD therapies are ineffective at stopping or even significantly slowing down the neurodegenerative processes; they only effectively cure symptoms. In order to assess the viability of a variety of medications with various pharmacological targets for the treatment of HD, numerous clinical trials have been completed and numerous others are in development. Very few drugs are FDA approved for the treatment of chorea. Therefore, a new phytochemicals with the highest potency and a specific target is needed. We are looking at the Neuroprotective Potential of Magnolol and Ursolic Acid in Animal Models of 3-Nitroproprionic Acid-Induced Huntington Disease for the first time.
- The study's findings are encouraging because they show that ursolic acid increases levels of the brain chemicals dopamine (DA), serotonin (5-HT), and gabaa and has a low affinity for the receptors for kainate, NMDA, and -amino-3-hydroxy-5-methylisoxazole proprionic acid (AMPA) while magnolol possesses the capability of penetrating the blood brain barrier. It may be helpful in maintaining the integrity of cell membranes of the Blood Brain Barrier whose permeability gets altered in neurodegenerative disorders like Huntington's, Parkinson's disease and Alzheimer's disease via membrane integrity agonist activity, As a free radical scavenger and anti-inflammatory, it may help shield neurons from the harmful effects of antioxidants and neuroinflammation.

OBJECTIVES

- To identify possible neuroprotective mechanisms of ursolic acid and magnolol, including antioxidant, anti-inflammatory actions and their association with the motor functions.
- To investigate both ursolic acid and magnolol effects on striatal monoaminergic transmission and their capacity to restore the glutamate and GABA levels to their normal patterns.
- To explore the involvement of nitric oxide pathway modulation in the protective effect of ursolic acid and magnolol.
- To carry out the Pharmacological screening (Neuroprotective, antioxidant & antidepressant) of ursolic acid and magnolol.

- To assess the synergistic effect of Ursolic acid and Magnolol with standard drug in Huntington's disease.

PROPOSED METHODOLOGY

The selected phytoactive chemical moiety will be examined for its potential to ward off Huntington's diseases by utilizing the methods specified in Table 1 (*In vivo* pharmacological investigations) and biochemical parameters to be evaluate are tabulated in Table 2.

The chemicals and instruments needed to conduct the pharmacological investigation are tabulated in Table 3 and Table 4 respectively. Keeping track of the study's observations, integrating the results with the statistical analysis the software tool to be used are presented in Table 5. A detail experimental design and plan of work with time line are tabulated in Table 6 and Table 7 respectively.

Table 1:- List of Models

Sr. No.	**Behavioral Parameters**
1.	Transfer Latency
2.	Escape Latency
3.	Locomotor Activity
4.	Probe Trial

Table 2: List of Analytical /Biochemical Parameters

Sr. No.	**Bio Chemical Estimations**
1.	Estimation of LDH
2.	Estimation of Total protein
3.	Estimation MDA (nmol/mg protein)
4.	Nitrite level (µg/ml)
5.	GSH (µmol GSH/ mg protein
6.	pro-inflammatory cytokines level
7.	TBARS

Table 3: List of Chemical

Chemicals Required		
Sr. No	**Name of Chemical**	**Amount required for 1000 ml**
1.	Sodium di-hydrogen phosphate	858 mg
2.	Sodium citrate	324 mg

3.	EDTA	2mg
4.	n-heptane sulphonic acid	45mg
5.	Acetonitrile	150ml
6.	di-Sodium hydrogen p hosphate	17.8 g
7.	Methanol	250 ml
8.	3-NP (3-nitropropionic acid)	0.2g
9.	Tris Hydrochloric acid (HCl)	100ml
10.	Hydrogen peroxide	Q.S.
11.	10% formalin	Q.S.
12.	Trichloroacetic acid (TCA)	Q.S.
13	Ursolic acid	1.8g
14	Magnolol	1.8g

Table 4: List of Instruments

Sr. No.	**Instrument / Equipment**
1.	UV-Visible Spectrophotometer
2.	Morris Water Maze
3.	Rotarod Appraatus
4.	Actophotometer
5.	Elevated Plus Maze
6.	Y-maze Test Apparatus
7.	pH Meter
8.	Sonicator
9.	Homogenizer
10.	Digital Balance
11.	Light Microscope
12.	Microtome
13.	Centrifuge
14.	Weighing Balance

Table 5:- List of Software's

Sr. No	**Software used in the study**
1.	Graph Pad Prism
2.	Instat

Histopathological Studies

Histopathological analysis will be carried out on decalcified sections (stained with Haematoxylin and Eosin) of brain samples cortex, striatum and hippocampus (will be

fixed in 10% neutral buffered formalin prior to emebedding in paraffin wax). The sections will be observed under light microscope for the microarchitectural changes.

Table 6: A Detail Experimental Design.

Groups	**Pharmacological Interventions**	**Duration**
Group-1	Normal Control (n=6)	21 Days
Group-2	Ursolic Acid perse; 40mg/kg/p.o (n=6)	21 Days
Group-3	Magnolol perse; 40mg/kg/p.o (n=6)	21 Days
Group-4	3-NP (10mg/kg) diseases control (n=6)	21 Days
Group-5	Standard dose (n=6) ; all animals will be observed for 21 Days	21 Days
Group-6	Ursolic acid low dose (20mg/kg/p.o) (n=6) + 3-NP (10mg/kg)	21 Days
Group-7	Ursolic acid high dose (40mg/kg/p.o) (n=6) + 3-NP (10mg/kg)	21 Days
Group-8	Magnolol low dose (20mg/kg/p.o) (n=6) + 3-NP (10mg/kg)	21 Days
Group-9	Magnolol high dose (40mg/kg/p.o) (n=6) + 3-NP (10mg/kg)	21 Days
Group-10	Ursolic acid (20 mg/kg; p.o) + Magnolol (20mg/kg;p.o) + 3-NP (10mg/kg) (n=6)	21 Days
Group-11	Ursolic acid (40 mg/kg; p.o) + Magnolol (40mg/kg;p.o) + 3-NP (10mg/kg) (n=6)	21 Days

Table 7: Plan of Work with Time Line

Phases	Research Work	Tentative Timeline for Purposed Work
Phase-1	• Literature Survey • Course Work	6 Months
	• Presentation on Topic Selection • Pre-Synopsis Presentation • Submission of Synopsis	6 Months
Phase-2	• Animal approval from CPCSEA. • Evaluate the Behavioral Parameters • Publication of one review paper in reputed journal	6 Months
	• Studying the effects of Ursolic acid and Magnolol on biochemical estimation and Histopathological changes in brain (cortex, striatum and hippocampus).	6 Months
	• In-vitro antioxidant activity by free radical scavenging, superoxide scavenging Assay of reducing power and DPPH radical scavenging activity.	
	• To represent my work in at least one national or international conference	
Phase-3	• Drafting of thesis and publication of at least two research papers in reputed journal. • Attend the one national or international conference	6 Months
	• Pre-Thesis Submission • Thesis Submission	6 Months

Statistical Analysis

All the values will be expressed as mean ± S.E.M. Data obtained from various groups will be assessed at Graph Pad Prism and statistically analyze using one way ANOVA, followed by Tukey test for comparison of all the groups.

THESIS OUTLINE

Chapter 1: INTRODUCTION. It covers the aim and objectives of research topic, i.e. Study of Possible Mechanisms Underlying In "Neuroprotective Potential of Ursolic Acid and Magnolol in 3-Nitroproprionic Acid-Induced Huntington Disease in Animal Model."

Chapter 2: LITERATURE REVIEW. It covers a review of work done on anti-oxidative activity as well as pharmacological effects of ursolic acid and magnolol on experimentally induced huntington's disease by the use of 3-NP

Chapter 3: MATERIALS AND METHODS. It covers the list of materials, equipment, and experimental protocols used in research work.

Chapter 4: RESULTS AND DISCUSSION. It covers the pharmacological evaluation of ursolic acid and magnolol.

Chapter 5: SUMMARY AND CONCLUSION. It covers the concise statement and key point of the neuroprotective mechanism.

Chapter 6: REFERENCES. It covers every source. The reference list entries contain four main components: author, date, title, and source.

REFERENCES

A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. (1993). *Cell*, *72*(6), 971–983. https://doi.org/10.1016/0092-8674(93)90585-e

Basso, M. (2012). Targeting transcriptional dysregulation in Huntington's disease: Description of therapeutic approaches. Huntington's disease—Core concepts and current advances (pp. 269–286).

Beal, M. F., & Ferrante, R. J. (2004). Experimental therapeutics in transgenic mouse models of Huntington's disease. *Nature Reviews. Neuroscience*, *5*(5), 373–384. https://doi.org/10.1038/nrn1386

Benito, E., & Barco, A. (2010). CREB's control of intrinsic and synaptic plasticity: Implications for CREB-dependent memory models. *Trends in Neurosciences*, *33*(5), 230–240. https://doi.org/10.1016/j.tins.2010.02.001

Binawade, Y., & Jagtap, A. (2013). Neuroprotective effect of lutein against 3-nitropropionic acid–induced Huntington's disease–like symptoms: Possible behavioral, biochemical, and cellular alterations. *Journal of Medicinal Food*, *16*(10), 934–943. https://doi.org/10.1089/jmf.2012.2698

Binienda, Z. K., Beaudoin, M. A., Gough, B., Ali, S. F., & Virmani, A. (2010). Assessment of 3-nitropropionic acid-evoked peripheral neuropathy in rats: Neuroprotective effects of acetyl-l-carnitine and resveratrol. *Neuroscience Letters*, *480*(2), 117–121. https://doi.org/10.1016/j.neul*et*2010.06.020

Bradford, J., Shin, J. Y., Roberts, M., Wang, C. E., Sheng, G., Li, S., & Li, X. J. (2010). Mutant huntingtin in glial cells exacerbates neurological symptoms of Huntington disease mice. *Journal of Biological Chemistry*, *285*(14), 10653–10661. https://doi.org/10.1074/jbc.M109.083287

Carlezon, W. A., Duman, R. S., & Nestler, E. J. (2005). The many faces of CREB. *Trends in Neurosciences*, *28*(8), 436–445. https://doi.org/10.1016/j.tins.2005.06.005

Chaturvedi, R. K., & Flint Beal, M. F. (2013). Mitochondrial diseases of the brain. *Free Radical Biology and Medicine*, 63, 1–29. https://doi.org/10.1016/j.freeradbiomed.2013.03.018

Checkoway, H., Lundin, J. I., & Kelada, S. N. (2011). Neurodegenerative diseases. *IARC Scientific Publications*, *163*(163), 407–419.

Chen, C. R., Tan, R., Qu, W. M., Wu, Z., Wang, Y., Urade, Y., & Huang, Z. L. (2011). Magnolol, a major bioactive constituent of the bark of Magnolia officinalis, exerts antiepileptic effects via the GABA/benzodiazepine receptor complex in mice. *British journal of pharmacology*, *164*(5), 1534–1546. https://doi.org/10.1111/j.1476-5381.2011.01456.x

Chen, J. Y., Wang, E. A., Cepeda, C., & Levine, M. S. (2013). Dopamine imbalance in Huntington's disease: A mechanism for the lack of behavioral flexibility. *Frontiers in Neuroscience*, *7*(114), 114. https://doi.org/10.3389/fnins.2013.00114

Costa, V., & Scorrano, L. (2012). Shaping the role of mitochondria in the pathogenesis of Huntington's disease. *EMBO Journal*, *31*(8), 1853–1864. https://doi.org/10.1038/emboj.2012.65

Dhir, A., Akula, K. K., & Kulkarni, S. K. (2008). Tiagabine, a GABA uptake inhibitor, attenuates 3-nitropropionic acid-induced alterations in various behavioral and biochemical parameters in rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *32*(3), 835–843. https://doi.org/10.1016/j.pnpbp.2007.12.017

DiFiglia, M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. P., & Aronin, N. (1997). Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science (New York, N.Y.), 277(5334), 1990–1993. https://doi.org/10.1126/science.277.5334.1990

Do, J., Kim, J. I., Bakes, J., Lee, K., & Kaang, B. K. (2012). Functional roles of neurotransmitters and neuromodulators in the dorsal striatum. *Learning and Memory*, *20*(1), 21–28. https://doi.org/10.1101/lm.025015.111

Ellerby, L. M. (2002). Hunting for excitement: NMDA receptors in Huntington's disease. *Neuron*, *33*(6), 841–842. https://doi.org/10.1016/s0896-6273(02)00631-1

Gusella, J. F., & MacDonald, M. E. (2009). Huntington's disease: The case for genetic modifiers. *Genome Medicine*, *1*(8), 80. https://doi.org/10.1186/gm80

Habtemariam S. (2019). Antioxidant and Anti-inflammatory Mechanisms of Neuroprotection by Ursolic Acid: Addressing Brain Injury, Cerebral Ischemia, Cognition Deficit, Anxiety, and Depression. *Oxidative medicine and cellular longevity*, *2019*, 8512048. https://doi.org/10.1155/2019/8512048

Hariharan, A., Shetty, S., Shirole, T., & Jagtap, A. G. (2014). Potential of protease inhibitor in 3-nitropropionic acid induced Huntington's disease like symptoms: Mitochondrial dysfunction and neurodegeneration. *Neurotoxicology*, *45*, 139–148. https://doi.org/10.1016/j.neuro.2014.10.004

Herculano-Houzel S. (2009). The human brain in numbers: a linearly scaled-up primate brain. *Frontiers in human neuroscience*, *3*, 31. https://doi.org/10.3389/neuro.09.031.2009

Hermann, B., & Seidenberg, M. (2007). Epilepsy and cognition. *Epilepsy Currents*, *7*(1), 1–6. https://doi.org/10.1111/j.1535-7511.2007.00151.x

Hong, J. M., Kim, J. E., Min, S. K., Kim, K. H., Han, S. J., Yim, J. H., Park, H., Kim, J. H., & Kim, I. C. (2021). Anti-Inflammatory Effects of Antarctic Lichen *Umbilicaria antarctica* Methanol Extract in Lipopolysaccharide-Stimulated RAW 264.7 Macrophage Cells and Zebrafish Model. *BioMed research international*, *2021*, 8812090. https://doi.org/10.1155/2021/8812090

Huang, L. S., Sun, G., Cobessi, D., Wang, A. C., Shen, J. T., Tung, E. Y., Anderson, V. E., & Berry, E. A. (2006). 3-nitropropionic acid is a suicide inhibitor of mitochondrial respiration that, upon oxidation by Complex II, forms a covalent adduct with a catalytic base arginine in the active site of the enzyme. *Journal of Biological Chemistry*, *281*(9), 5965–5972. https://doi.org/10.1074/jbc.M511270200

Huitron, R. L., Muniz, P. U., Pineda, B., Chaverrí, J. P., Ríos, C., & Cruz, V. P. (2013). Quinolinic acid: An endogenous neurotoxin with multiple targets. *Oxidative Medicine and Cellular Longevity*, *2013*, 1–14.

Iennaco, R., Formenti, G., Trovesi, C., Rossi, R. L., Zuccato, C., Lischetti, T., Bocchi, V. D., Scolz, A., Martínez-Labarga, C., Rickards, O., Pacifico, M., Crottini, A., Moller, A. P., Chen, R. Z., Vogt, T. F., Pavesi, G., Horner, D. S., Saino, N., & Cattaneo, E. (2022). The evolutionary history of the polyQ tract in huntingtin sheds light on its functional pro-neural activities. Cell Death and Differentiation, 29(2), 293–305. https://doi.org/10.1038/s41418-021-00914-9

Johri, A., & Beal, M. F. (2012). Antioxidants in Huntington's disease. *Biochimica et Biophysica Acta*, *1822*(5), 664–674. https://doi.org/10.1016/j.bbadis.2011.11.014

Kandel, E. R. (2012). The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. *Molecular Brain, 5*, 14. https://doi.org/10.1186/1756-6606-5-14

Khan, A., Jamwal, S., Bijjem, K. R. V., Prakash, A., & Kumar, P. (2015). Neuroprotective effect of hemeoxygenase-1/glycogen synthase kinase-3β modulators in 3-nitropropionic acid-induced neurotoxicity in rats. *Neuroscience*, *287*, 66–77. https://doi.org/10.1016/j.neuroscience.2014.12.018

Kim, G. W., Copin, J. C., Kawase, M., Chen, S. F., Sato, S., Gobbel, G. T., & Chan, P. H. (2000). Excitotoxicity is required for induction of oxidative stress and apoptosis in mouse striatum by the mitochondrial toxin, 3-nitropropionic acid. *Journal of Cerebral Blood Flow and Metabolism*, *20*(1), 119–129. https://doi.org/10.1097/00004647-200001000-00016

Kim, S. H., Thomas, C. A., André, V. M., Cummings, D. M., Cepeda, C., Levine, M. S., & Ehrlich, M. E. (2011). Forebrain striatal-specific expression of mutant huntingtin protein in vivo induces cell-autonomous age-dependent alterations in sensitivity to excitotoxicity and mitochondrial function. ASN Neuro, 3(3), e00060. https://doi.org/10.1042/AN20110009.

Kumar, P., & Kumar, A. (2009). Neuroprotective effect of cyclosporine and FK506 against 3-nitropropionic acid induced cognitive dysfunction and glutathione redox in rat: Possible role of nitric oxide. *Neuroscience Research*, *63*(4), 302–314. https://doi.org/10.1016/j.neures.2009.01.005

Kumar, P., Kalonia, H., & Kumar, A. (2011). Novel protective mechanisms of antidepressants against 3-nitropropionic acid induced Huntington's-like symptoms: A comparative study. *Journal of Psychopharmacology*, *25*(10), 1399–1411. https://doi.org/10.1177/0269881110364269

Kushner, D. (1998). Mild traumatic brain injury: Toward understanding manifestations and treatment. *Archives of Internal Medicine*, *158*(15), 1617–1624. https://doi.org/10.1001/archinte.158.15.1617

Lee, Y. W., Cho, H. J., Lee, W. H., & Sonntag, W. E. (2012). Whole brain radiation-induced cognitive impairment: Pathophysiological mechanisms and therapeutic targets. *Biomolecules and Therapeutics*, *20*(4), 357–370. https://doi.org/10.4062/biomolther.2012.20.4.357

Li, Q., Fan, Y. S., Gao, Z. Q., Fan, K., & Liu, Z. J. (2015). Effect of Fructus Ligustri Lucidi on osteoblastic like cell-line MC3T3-E1. *Journal of ethnopharmacology*, *170*, 88–95. https://doi.org/10.1016/j.jep.2015.05.013

López-Hortas, L., Pérez-Larrán, P., González-Muñoz, M. J., Falqué, E., & Domínguez, H. (2018). Recent developments on the extraction and application of ursolic acid.

A review. *Food Research International*, *103*, 130–149. https://doi.org/10.1016/j.foodres.2017.10.028

Luchsinger, J. A., & Mayeux, R. (2004). Dietary factors and Alzheimer's disease. *Lancet Neurology*, *3*(10), 579–587. https://doi.org/10.1016/S1474-4422(04)00878-6

Ma, J. Q., Ding, J., Zhang, L., & Liu, C. M. (2014). Ursolic acid protects mouse liver against CCl4-induced oxidative stress and inflammation by the MAPK/NF-κB pathway. *Environmental Toxicology and Pharmacology*, *37*(3), 975–983. https://doi.org/10.1016/j.etap.2014.03.011

Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trottier, Y., Lehrach, H., Davies, S. W., & Bates, G. P. (1996). Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell*, *87*(3), 493–506. https://doi.org/10.1016/s0092-8674(00)81369-0

Mark, L. P., Prost, R. W., Ulmer, J. L., Smith, M. M., Daniels, D. L., Strottmann, J. M., Brown, W. D., & Hacein-Bey, L. H. (2001). Pictorial review of glutamate excitotoxicity: Fundamental concepts for neuroimaging. *AJNR. American Journal of Neuroradiology*, *22*(10), 1813–1824.

Mehan, S., Nain, V. S., Meena, H., Sharma, D., Sankhla, R., & Singh, M. (2010). Comparative effect of phosphodiesterase inhibitors on intracerebroventricular cochicine model of memory impairment in rats. *International Journal of Pharma Professional's Research*, *1*(2), 122–136.

Meng, Y. Q., Xu, C. D., Yu, T. T., Li, W., Li, Q. W., & Li, X. X. Synthesis and antitumor activity evaluation of ursolic acid derivatives. (2020). *Journal of Asian Natural Products Research*, 22(4), 359–369. https://doi.org/10.1080/10286020.2019.1571488

Milnerwood, A. J., Gladding, C. M., Pouladi, M. A., Kaufman, A. M., Hines, R. M., Boyd, J. D., Ko, R. W., Vasuta, O. C., Graham, R. K., Hayden, M. R., Murphy, T. H., & Raymond, L. A. (2010). Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. *Neuron*, *65*(2), 178–190. https://doi.org/10.1016/j.neuron.2010.01.008.

Mlala, S., Oyedeji, A. O., Gondwe, M., & Oyedeji, O. O. (2019). Ursolic acid and its derivatives as bioactive agents. *Molecules*, *24*(15), 2751.

Morris, M. S., Jacques, P. F., Rosenberg, I. H., & Selhub, J. (2007). Folate and vitamin B-12 status in relation to anemia, macrocytosis and cognitive impairment in older

Americans in the age of folic acid fortification. *American Journal of Clinical Nutrition*, *85*(1), 193–200. https://doi.org/10.1093/ajcn/85.1.193

Mutafova-Yambolieva, V. N., Smyth, L., & Bobalova, J. (2003). Involvement of cyclic AMP-mediated pathway in neural release of noradrenaline in canine isolated mesenteric artery and vein. *Cardiovascular Research*, *57*(1), 217–224. https://doi.org/10.1016/s0008-6363(02)00648-x

Nagakura, A., Niimura, M., & Takeo, S. (2002). Effects of a phosphodiesterase IV inhibitor Rolipram on microsphere embolism-induced defects in memory function and cerebral cyclic AMP signal transduction system in rats. *British Journal of Pharmacology*, *135*(7), 1783–1793. https://doi.org/10.1038/sj.bjp.0704629

Pandey, M., Borah, A., Varghese, M., Barman, P. K., Mohanakumar, K. P., & Usha, R. (2009). Striatal dopamine level contributes to hydroxyl radical generation and subsequent neurodegeneration in the striatum in 3-nitropropionic acid-induced Huntington's disease in rats. *Neurochemistry International*, *55*(6), 431–437.

Puzzo, D., Vitolo, O., Trinchese, F., Jacob, J. P., Palmeri, A., & Arancio, O. (2005). Amyloid-beta peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element-binding protein pathway during hippocampal synaptic plasticity. *Journal of Neuroscience*, *25*(29), 6887–6897. https://doi.org/10.1523/JNEUROSCI.5291-04.2005

Ribeiro, F. M., Dobransky, T., Carvalho, E. A., Cruz, J. S., & Oliveira, F. A. (2012). *Energy metabolism in Huntington's disease. Huntington's disease—Core concepts and current advances* (pp. 323–346).

Safayhi, H., & Sailer, E. R. (1997). Anti-inflammatory actions of pentacyclic triterpenes. *Planta medica*, *63*(6), 487–493. https://doi.org/10.1055/s-2006-957748

Scott Bitner, R. S. (2012). Cyclic AMP response element-binding protein (CREB) phosphorylation: A mechanistic marker in the development of memory enhancing Alzheimer's disease therapeutics. *Biochemical Pharmacology*, *83*(6), 705–714. https://doi.org/10.1016/j.bcp.2011.11.009

Shivasharan, B. D., Nagakannan, P., Thippeswamy, B. S., Veerapur, V. P., Bansal, P., & Unnikrishnan, M. K. (2013). Protective effect of Calendula officinalis Linn. flowers against 3-nitropropionic acid induced experimental Huntington's disease in rats. *Drug and Chemical Toxicology*, *36*(4), 466–473. https://doi.org/10.3109/01480545.2013.776583

Swaroop, T. V., Banerjee, S., & Handral, M. (2014). Neuroprotective Evaluation of Leaf Extract of Dalbergia sissoo in 3-nitropropionic Acid Induced Neurotoxicity in Rats. *International Journal of Pharmaceutical Sciences and Drug Research*, *6*(1), 41–47.

Túnez, I., Tasset, I., Pérez-De La Cruz, V., & Santamaría, A. (2010). 3-Nitropropionic acid as a tool to study the mechanisms involved in Huntington's disease: past, present and future. *Molecules (Basel, Switzerland)*, *15*(2), 878–916. https://doi.org/10.3390/molecules15020878

Uzun, S., Kozumplik, O., Jakovljevic, M., & Sedic, B. (2010). Side effects of treatment with benzodiazepines. *Psychiatria Danubina*, *22*(1), 90–93.

Vogelsberg, V., Neff, N. H., & Hadjiconstantinou, M. (1997). Cyclic AMP Mediated enhancement of high affinity choline Transport and Achetylcholine synthesis in brain. *J. Neurochein*, *68*(3), 1062–1070.

Wang, L., Wang, J., Yang, L., Zhou, S. M., Guan, S. Y., Yang, L. K., Shi, Q. X., Zhao, M. G., & Yang, Q. (2017). Effect of Praeruptorin C on 3-nitropropionic acid induced Huntington's disease-like symptoms in mice. *Biomedicine and Pharmacotherapy*, *86*, 81–87. https://doi.org/10.1016/j.biopha.2016.11.111

Zhang, J., Wang, Q., Zhu, N., Yu, M., Shen, B., Xiang, J., & Lin, A. (2008). Cyclic AMP inhibits JNK activation by CREB-mediated induction of c-FLIP(L) and MKP-1, thereby antagonizing UV-induced apoptosis. *Cell Death and Differentiation*, *15*(10), 1654–1662. https://doi.org/10.1038/cdd.2008.87

Zhu, S., Liu, F., Zhang, R., Xiong, Z., Zhang, Q., Hao, L., & Chen, S. (2022). Neuroprotective Potency of Neolignans in *Magnolia officinalis* Cortex Against Brain Disorders. *Frontiers in pharmacology*, *13*, 857449. https://doi.org/10.3389/fphar.2022.857449

Zwolak, I. (2014). Vanadium carcinogenic, immunotoxic and neurotoxic effects: A review of in vitro studies. Toxicology Mechanisms and Methods, 24(1), 1–12. https://doi.org/10.3109/15376516.2013.843110.

CERTIFICATE

(SYNOPSIS APPROVAL CERTIFICATE)

This is to certify that the synopsis for Ph.D. Thesis titled, **"Study of Possible Mechanism Underlying in Neuroprotective Potential of Ursolic Acid and Magnolol in 3-Nitropropionic Induced Huntington Disease in Animal Model",** submitted by **Mr. Ravinder Khatri** to the Career Point University, Hamirpur (H.P.), India in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in the **Department of Pharmaceutical Sciences, School of Pharmaceutical and Health Sciences** has been approved by the Research Advisory Committee (RAC) after an Oral Examination of the student on dated 15/05/2023.

(Supervisor)
Dr. Kamal Jeet
Assitant Professor
Department of Pharmacy

Research Advisory Committee

Dr. Vijay Kumar
Assitant Professor
Division of Microbilogy

Dr. Arti Jamwal
Assitant Professor
Department of Bioscience

Dr. Anjana Devi
(Head)
Department of Pharmacy

Dr. Kuldeep Kumar
(Dean/Assoc Dean Research & Development)

CERTIFICATE

(SYNOPSIS APPROVAL CERTIFICATE)

This is to certify that the synopsis for Ph.D. Thesis titled, **"Study of Possible Mechanism Underlying in Neuroprotective Potential of Ursolic Acid and Magnolol in 3-Nitropropionic Induced Huntington Disease in Animal Model"**, submitted by **Mr. Ravinder Khatri** to the Career Point University, Hamirpur (H.P.), India in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in the **Department of Pharmaceutical Sciences, School of Pharmaceutical and Health Sciences** has been approved by the Research Advisory Committee (RAC) after an Oral Examination of the student on dated 15/05/2023.

(Supervisor)
Dr. Kamal Jeet
Assistant Professor
Department of Pharmacy

Research Advisory Committee

Dr. Vijay Kumar
Assistant Professor
Division of Microbiology

Dr. Arti Jamwal
Assistant Professor
Department of Bioscience

Dr. Anjana Devi
(Head)
Department of Pharmacy

Dr. Kuldeep Kumar
(Dean/Assoc Dean Research & Development)

Scanned with OKEN Scanner